# Studies on pollen morphology of selected species of Annonaceae from Thailand

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## Abstract

The pollen morphology of 48 species and two varieties, representing 18 genera of the family Annonaceae from Thailand, is described and illustrated based on observations with scanning electron microscopy (SEM). The results reaffirm the great diversity in Annonaceae at the pollen morphological level, with the size of pollen varying from small, medium-sized to large, the shape of monads being elliptic, spheroidal or triangular with monosulcate, disulculate or no visible aperture, and the shape of tetrads being tetragonal, rhomboidal, tetrahedral, T-shaped or decussate. Ornamentation is echinate, psilate, microreticulate, rugulate, scabrate or verrucate. Decussate tetrads in *Goniothalamus*, and tetragonal, rhomboidal, T-shaped and decussate tetrads and new tetrad type in *Mitrephora* are first reported. The pollen grains of *Anaxagorea* show slightly bilateral and no visible aperture as a result of intine extrusion. The palynological evidence confirms the close relationship of *Dasymaschalon*, *Desmos* and Asian *Friesodielsia*, and shows the high diversity of pollen morphology within genera in Annonaceae.

**Keywords**   Annonaceae, diversity, morphology, pollen

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## Introduction

The pantropical flowering plant Annonaceae comprise ca. 108 genera and ca. 2300 species of trees, shrubs and lianas (Rainer & Chatrou 2006, Chatrou et al. 2012), and show three continental grouping (Asia, America and Africa) of equivalent size (Fries 1959; Keβler 1993). Being the largest family in the early-divergent Magnoliales (Massoni et al. 2014; Angiosperm Phylogeny Group 2016), Annonaceae are noteworthy for having greatest diversity in the order not only at the macromorphological level (Silberbauer-Gottsberger et al. 2003; Gottsberger 2012), but also have the most diverse pollen morphology (Walker 1971a, b, c; Le Thomas 1980, 1981; Doyle & Le Thomas 2012).

Since the 1970s, many researches regarding to pollen characters in Annonaceae have been carried out, and the results exhibited the high pollen diversity which were also applied in clarifying high-level relationships. The pollen morphology of tropical American Annonaceae was extensively studied with the application of light microscopy (LM) and scanning electron microscopy (SEM), and was used as the primary basis for a classification of the family into three subfamilies and seven tribes (Walker 1971a, 1971b, 1971c, 1972). The pollen ultrastructure of Africa Annonaceae was comprehensively investigated by Le Thomas (1980, 1981), and the results elucidated the value of the pollen characters in taxonomy and phylogeny of the family. More details of pollen structure were examined by Waha and Morawetz (1988), Su and Saunders (2003), Chaowasku et al. (2008), Couvreur et al. (2008), Xu and Craene (2013) and Gan et al. (2015). Pollen wall development was also studied by Gabarayeva (1993, 1995), Waha (1987), and Lora et al. (2009). Doyle. (Doyle et al 2000) and Le Thomas (Doyle et??1994, 1997, 2012) used molecular analyses to present the evolution and phylogenetic value of pollen in Annonaceae, and confirmed the significance of pollen in recognizing major groups and their relationship

The molecular analyses have been widely applied in solving phylogeny and evolution problems (Mols et al. 2004, 2008; Xue et al. 2011; Chatrou et al. 2012; Li et al. 2015; Mercer et al. 2016). However, the successes of molecular phylogenetics do

not mean there is no longer any reason to study morphology (Doyle & Le Thomas 2012). In Annonaceae, the high diverse pollen morphology is more reliable than flower morphology in higher-level systematics, and further investigations on pollen morphology are still needed (Mols et al. 2004, 2008). In spite of massive researches on Annonaceae pollen, the pollen characters of many species only have LM data and a host of pollen photographs are not in good quality. Although the pollen morphology of the America and Africa Annonaceae has been extensively studied ((Walker 1971a, 1971b, 1971c; Le Thomas 1980, 1981), that of Asia Annonaceae has been relatively poorly investigated, especially in Thailand, where the Annonaceae plants widely distribute. The aim of the present study is to provide a more detailed description on the pollen morphology of Thai Annonaceae, and offer some palynological evidence to better understand the pollen diversityand the phylogenetic significant of the pollen characters.

**Materials and methods**

48 species and two varieties, representing 18 genera of Annonaceae were investigated. Flowers were collected from October 2014 to April 2015 by Dr. Piya Chalermglin, Institute of Scientific and Technological Research, Thailand and fixed in formalin acetic alcohol (FAA: 70% alcohol, formaldehyde and glacial acetic acid in a ratio of 90:5:5). Pollen grains were collected from mature and indehiscent stamens.

Pollen grains of many Annonaceae are very fragile, and the acetolysis method for pollen preparation (Erdtman 1960) is often too drastic and damages the pollen grains making observations difficult. An alternative method based on mature stamens was used. The fixed stamens were dehydrated in ethylalcohol ascending series, critical-point dried. The pollen sacs of these mature stamens were opened carefully with a tweezer and needle in order to release the pollen grains from the sacs. Pollen grains destined for SEM were placed directly on brass stubs without treatment, coated with gold using a JFC-1600 Auto Fine Coater and observed with a JSM-6360LV (JEOL, Tokyo, Japan) scanning electron microscope operated at 25kV. More than 10

pollen grains were examined.

The lengths of the long axis (LA) and the short axis (SA) were measured from at least 20 mature pollen grains of each species using LM slides of pollen mounted in glycerin (Xu & Kirchoff 2008). All photographs were edited by Adobe Photoshop CS4. Descriptive palynological terminology follows that of Punt et al. (2007). Details for each species ranked alphabetically are given in Table I and II.

**Results**

The LA of pollen grains examined is 22–123 μm, and the SA is 15–97 μm. Most examined pollen grains are monads with spheroidal, elliptic or triangular shape. Monads with one (or two) furrow(s) or depressed area(s) were discovered in *Anaxagorea*, *Artabotrys*, *Huberantha*, *Marsypopetalum*, *Miliusa*, *Orophea* and *Polyalthia*. Others are spheroidal or elliptic with no visible aperture or indication of a pole. Tetrads were observed in *Goniothalamus* (Figures 2O, Q, S, 3A, C) and *Mitrephora* (Figures 3Q–T, 4B–E, G–I, K–M, O–S, 5A–C, E–G), including tetragonal, rhomboidal, T-shaped, tetrahedraland decussate. The echinate, psilate, scabrate, reticulate, rugulate and verrucate ornamentations were also observed.

*Alphonsea* Hook. f. et Thomson

*Alphonsea pallida* and *A. siamensis* were investigated. The pollen grains are spheroidal monads with no visible aperture or indication of a pole (Figure 1A, C). The exine ornamentation is rugulate (Figure 1B, D). The mean LA of the pollen grains is 34 μm in *A. pallida* and 36 μm in *A. siamensis*.

*Anaxagorea* St. Hil.

*Anaxagorea javanica* and *A. luzonensis* were investigated. The pollen grains are slightly bilateral and elliptic monads with no visible aperture (Figure 1E, G). The exine ornamentation is psilate in *A. javanica*, while scabrate and psilate in *A. luzonensis* (Figure 1F, H). The mean LA of the pollen grains is 70 μm in *A. javanica*, and 72 μm in *A. luzonensis*.

*Artabotrys* R. Br.

Six species were investigated. The pollen grains are elliptic monads (Figure 1I, K, M, O, Q, S) with a single furrow in *A. burmanicus* (Figure 1K) and with no visible aperture or indication of a pole in *A. brevipes*, *A. harmandii*, *A. multiflorus*, *A. siamensis* and *A. uniflorus* (Figure 1I, M, O, Q, S). The exine ornamentation is loosely fossulate-perforate in *A. brevipes and A. harmandii* (Figure 1J, N), finely fossulate-perforate in *A. burmanicus*, *A. multiflorus*, *A. siamensis* and *A. uniflorus* (Figure 1L, R, T, P). The mean LA of the pollen grains is 48 μm in *A. brevipes* and *A. siamensis*, 50 μm in *A. burmanicus* and *A. harmandii*, 34 μm in *A. multiflorus*, 42 μm in *A. uniflorus*.

*Dasymaschalon* Dalla Torre et Harms

Four species were investigated. The pollen grains are spheroidal monads with no visible aperture or indication of a pole (Figure 2A, C, E, G). The exine ornamentation is echinate with much smaller, closely spaced clavae between the spins in *D. acuminatum* and *D. angustifolium* (Figure 2B, D), echinate with many shallows in *D. macrocalyx* (Figure 2H), and scabrate in *D. dasymaschalum* (Figure 2F). The mean LA of the pollen grains is 35 μm in *D. acuminatum* and *D. macrocalyx*, 34 μm in *D. angustifolium*, and 22 μm in *D. dasymashalum*.

*Desmos* Lour.

The pollen grains of *Desmos cochinchinensis* are spheroidal monads with no visible aperture or indication of a pole (Figure 2I). The exine ornamentation is echinate with well-developed spines (Figure 2J). The mean LA of the pollen grains is 28 μm.

*Fissistigma* Griff.

The pollen grains of *Fissistigma oblongum* are elliptical monads with a distinctly flattened (probably proximal) pole (Figure 2K). The exine ornamentation is microrugulate (Figure 2L). The mean LA of the pollen grains is 35 μm.

*Friesodielsia* Steenis

The pollen grains of *Friesodielsia desmoides* are spheroidal monads with no visible aperture or indication of a pole (Figure 2M). The exine ornamentation is echinate-perforate with well-developed spines (Figure 2N). The mean LA of the pollen grains is 29 μm.

*Goniothalamus* (Blume) Hook. f. et Thomson

Five species were investigated. The pollen grains are tetrads with no visible aperture. The tetrads are tetrahedral in *G. laoticus* (Figure 2O), decussate in *G. repevensis* (Figure 2Q), and tetragonal in *G. sawtehii* (Figure 2S), *G. tamiresis* (Figure 3A) and *G. undulatus* (Figure 3C), The exine ornamentation is psilate in *G. laoticus* (Figure 2P) and *G. tamirensis* (Figure 3B), psilate-perforate in *G. repevensis* (Figure 2R), rugulate in *G. sawtehii* (Figure 2T) and coarsely scabrate in *G. undulatus* (Figure 3D). The mean LA of the pollen grains is 105 μm in *G. laoticus* and *G. repevensis*, 77 μm in *G. sawtehii*, 123 μm in *G. tamirensis*, and 80 μm *G. undulatus*.

*Huberantha* Chaowasku

The pollen grains of *Huberantha cerasoides* are spheroidal monads with two depressed areas (Figure 3E). The exine ornamentation is rugulate-verrucate (Figure 3F). The mean LA of the pollen grains is 28 μm.

*Marsypopetalum* Scheff.

The pollen grains of *Marsypopetalum crassum* are elliptic disulculate monads (Figure 3G). The exine ornamentation is shallowly verrucate (Figure 3H). The mean LA of the pollen grains is 37 μm.

*Melodorum* Lour.

The pollen grains of *Melodorum fruticosum* are elliptic monads with no visible aperture or indication of a pole (Figure 3I). The exine ornamentation is scabrate-perforate (Figure 3I). The mean LA of the pollen grains is 43 μm.

*Miliusa* Lesch. ex A. DC.

Three species were investigated. The pollen grains are spheroidal monads with two depressed areas (Figure 3 K, M, O). The exine ornamentation is verrucate-perforate in *M. campanulata* (Figure 3L) and *M. cuneata* (Figure 3N), and scabrate in *M. intermedia* (Figure 3P). The mean LA of the pollen grains is 40 μm in *M. campanulata*, and 34 μm in *M. cuneata* and *M. intermedia*.

*Mitrephora* (Blume) Hook. f. et Thomson

Seven species were investigated. The pollen grains are tetrads with no visible aperture. The tetrads are tetragonal in *M. alba*, *M. keithii*, *M. sp.*, *M. teysmanii*, *M. tomentosa*,

*M. wangii* and *M. winitii* (Figures 3Q, 4B, G, K, O, 5A, E), rhomboidal in *M. alba*, *M. keithii*, *M. sp.*, *M. teysmanii*, *M. tomentosa*, *M. wangii* and *M. winitii* (Figures 3R, 4C, H, L, P, 5B, F), T-shaped in *M. tomentosa* (Fig. 4Q), tetrahedral in *M. alba*, *M. keithii*, *M. sp.*, *M. teysmanii*, *M. tomentosa* and *M. winitii* (Figures 3S, 4D, I, M, R, 5G), decussate in *M. alba* and *M. tomentosa* (Figures 3T, S) and a new tetrad type (a multiplanar tetrad of pollen grains arranged in two pairs, and the pairs contact with each other at one side, but not at right angles) *M. keithii* (Figures 4E). The exine ornamentation is verrucate (Figures 4A, F, J, N, T, 5D, H). The mean LA of the pollen grains is 63 μm in *M. alba*, *M. keithii*, *M. tomentosa* and *M. winitii*, 47 μm in *M. sp.*, 50 μm in *M. teysmanii*, and 70 μm in *M. wangii*.

*Monoon* Miq.

Six species were investigated. The pollen grains are spheroidal monads with no visible aperture or indication of a pole (Figure 5I, K, M, O, Q, S). The exine ornamentation is shallowly microechinate in *M*. *asteriellum* (Figure 5J), scabrate in *M. fuscum* and *M. membranifolium* (Figure 5L, R), rugulate in *M. jucundum*, *M. lateriflorum* and *M. viride* (Figure 5N, P, T). The mean LA of the pollen grains is 23 μm in *M. asteriellum*, 40 μm in *M. fuscum*, 32 μm in *M. jucundum* and *M. lateriflorum*, 28 μm in *M. membranifolium* and *M. viride*.

*Orophea* Blume

Four species were investigated. The pollen grains are spheroidal monads with two (or one) furrows (furrow) (Figure 6A, C, E, G). The exine ornamentation is verrucate in *O*. *brandisii* and *O. enterocarpa* (Figure 6B, D), rugulate in *O. kerrii*, and reticulate-perforate in *O. polycarpa* (Figure 6F, H). The mean LA of the pollen grains is 45 μm in *O. brandisii*, 40 μm in *O. enterocarpa*, 32 μm in *O. kerrii*, and 44 μm in *O. polycarpa*.

*Polyalthia* Blume

Two species, one including two varieties were investigated. The pollen grains are triangular disulculate monads in *P. bullata* (Figure 6I, J) or with no visible aperture or indication of a pole in *P. evecta* var. *attopeuernsi* (Figure 6N, O), and spheroidal monosulcate monads in *P. evecta* var. *evecta* (Figure 6L). The exine ornamentation is

rugulate (Figure 6K) in *P. bullata*, verrucate in *P. evecta* var. *evecta* (Figure 6M) and rugulate-verrucate in *P. evecta* var. *attopeuernsi* (Figure 6P). The mean LA of the pollen grains is 48 μm in *P. bullata*, 41 μm in *P. evecta* var. *evecta*, and 50 μm in *P. evecta* var. *attopeuernsis*.

*Uvaria* L.

The pollen grains of *Uvaria siamensis* are spheroidal monads with no visible aperture or indication of a pole (Figure 6Q). The exine surface is gemmate (Figure 6R). The mean LA of the pollen grains is 48 μm.

*Winitia* Chaowasku

The pollen grains of *Winitia expansa* are elliptic monads with no visible aperture (Figure 6S). The exine surface is microscabrate (Figure 6T). The mean LA of the pollen grains is 23 μm.


**Discussion**


The pollen morphology of Annonaceae is highly variable in the shape, size, pollen unit, exine ornamentation and aperture number.

The molecular analyses confirmed the earliest diverging position of *Anaxagorea*, which is sister to the remainder of the family (Chatrou et al. 2012). Likewise, the granular monosulcate pollen of *Anaxagorea* was regarded as an ancestral type in Annonaceae (Doyle & Le Thomas 1994, 1996, 2012). However, in the present study pollen of *A. javanica* and *A. luzonensis* are slightly bilateral, and two different kind of exine ornamentation occurring in one pollen grain , which is inconsistent with the boat-like shape and granular monosulcate *Anaxagorea* pollen in previous investigations oyle & Le Thomas 1994, 1996, 2012). The most likely reason for the inconsistence is that the intine extrudes from exine through the aperture, and that is also the reason why no visible aperture was observed, while the exinous margins of sulcus were clearly demonstrated. The intine extrusion is considered as a typical phenomenon in *Anaxagorea* species (Hess et al. 1985). In the present study, it is not easy to distinguish exine and intine in SEM micrograph of *A. javanica* because they

have similar surfaces. In *A. luzonensis*, the scabrate hemisphere is exine and the psilate hemisphere is intine. The boat-shaped pollen in previous studies might be a result of the acetolysis method, which sometimes led to the misinterpretation in respect to pollen shape and aperture form (Hess et al. 1985).

The close relationship of *Dasymaschalon*, *Desmos* and Asian *Friesodielsia* is proved by molecular studies (Richardson et al. 2004; Couvreur et al. 2011; Wang et al. 2009, 2012). In previous investigations, *Dasymaschalon*, *Desmos* and Asian *Friesodielsia* usually have inaperturate pollen with echinate ornamentation (Walker 1971; Le Thomas 1980, 1981; Bygrave 2000; Doyle & Le Thomas 2012). The present study was basically consistent with the previous reports but some minor differences were detected. In *Dasymaschalon acuminatum* and *D. angustifolium*, the ornamentation is echinate with intervening smaller and thinner clavae, in *D. macrocalyx* echinate with many shallows and in *Desmos cochinchinensis* and *Friesodielsia desmoides* echinate, respectively. Despite of the minor differences, the inaperturate pollen with echinate ornamentation is quite stable. Inaperture and echinate ornamentation could probably be considered as a synapomorphy of *Dasymaschalon*, *Desmos* and Asian *Friesodielsia*.

The pollen grains of *Goniothalamus* were reported to have tetragonal or tetrahedral tetrads (Walker 1971a; Gan et al. 2015). In the present investigation, three tetrad types (tetragonal, tetrahedral and decussate) were observed, and the decussate tetrads were reported for the first time in *Goniothalamus*.　And also psilate-perforate (*G. repevensis*), rugulate (*G. sawtehii*) and scabrate ornamentation (*G. undulatus*) patterns were observed in this study, whereas only psilate ornamentation was reported previously (Walker 1971a).

The pollen grains of studied *Mitrephora* species were released as tetrahedral tetrads (Walker 1971a; Weerasooriya 2001; Weerasooriya & Saunders 2010). However, five tetrad types (tetragonal, rhomboidal, tetrahedral, decussate and T-shaped) were observed in *Mitrephora* in the present investigation, even in one species (*M. tomentosa*).A new tetrad type, which is likely formed by two dyads and the two contact areas of dyads are perpendicular, was also observed in *M. keithii*. The

various tetrad types in *Goniothalamus* and *Mitrephora* confirm the diversity of pollen morphology within genera in Annonaceae. It is important to investigate much more to evaluate the taxonomic significance of pollen morphology

Among taxa now assigned to the tribe Miliuseae, *Polyalthia* in its traditional broad sense would be the largest genus? (Chatrou et al. 2012). Early studies showed that *Polyalthia* was highly varied palynologically (Le Thomas 1980, 1981), and was considered to be polyphyletic (Doyle & Le Thomas 1994, 1996; Doyle et al. 2000; Chatrou et al. 2012). Based on molecular phylogenetic analyses and morphological evidence, the large, heterogeneous genus *Polyalthia* has been segregated into several smaller monophyletic genera, and the species in this study has been classified into four genera :*Huberantha*, *Marsypopetalum*, *Monoon* and *Polyalthia* s.str (Xue et al. 2011, 2012; Chaowasku et al. 2012, 2015). In this investigation, the species of *Huberantha* and *Marsypopetalum*, and four species (*M. asteriellum, M. jucundum M. lateriflorum* and *M. viride*) of *Monoon* are transferred from *Polyalthia* s.l. (Xue et al. 2011, 2012; Chaowasku et al. 2012). Pollen aperture seems to be rather consistent within *Huberantha*, *Marsypopetalum* and *Monoon*, with two depressed areas in *Huberantha*, disulculate in *Marsypopetalum* but inaperturate in *Monoon*, respectively. The two depressed areas in *H. cerasoides* in the present study are similar to that of *H. pendula* , of which was considered astwo germination zones. (Chaowasku et al. 2008, 2012). *Marsypopetalum crassum*, treated as synonym of *M. modestum* (Turner 2015), has disulculate pollen, which is consistent in *Marsypopetalum* and common with its sister genus, *Trivalvaria*. The genus *Monoon* currently consists of 37 *Polyalthia* s.l. species, all species of *Enicosanthum* and *Woodiellantha sympetala*. Except for the four *Polyalthia* s.l. species, *M. fuscum* and *M. membranifolium* in this work belonged to *Enicosanthum* (Xue et al. 2012). The six *Monoon* species all exhibit inaperturate monads, which is similar to the pollen of *Enicosanthum* and *Woodiellantha,* described by Walker (1971a).

. In the present investigation, only two species, (including two varieties) were observed but pollen aperture morphology in *Polyalthia* s.str. demostrates diversity, since inaperturate, monosulcate and disulculate pollen with rugulate, verrucate or

rugulate-verrucate ornamentation appeared. At the same time, both triangular and spheroidal monads were observed. Although, in *Polyalthia* s.str pollen morphology has high diversity, it is helpful in elucidating the phylogenetic affinities within genera, because the recent classification was established basing on the result of molecular analyses and morphology, largely focusing on pollen chacracters (Saunders et al. 2011).n..

## Conclusions

The pollen morphology of Annonaceae exhibits high diversity in this study. Pollen appears slightly bilateral in *Anaxagorea* because of the intine extrusion. The palynological results presented here support the close relationship of *Dasymachalon*, *Desmos* and Asian *Friesodielsia*. The coexistence of five different tetrad types in *Mitrephora tomentosa* and new tetrad type in M. keithii are first reported. The various tetrad types in *Goniothalamus* and *Mitrephora* and pollen in *Polyalthia* confirm the high diversity within genera at the pollen morphological level in Annonaceae. Additional work is necessary to evaluate the significance of tetrads types in species identification in *Goniothalamus* and *Mitrephora*, and to test the status of the large genus *Polyalthia* s.str..

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## Disclosure statement

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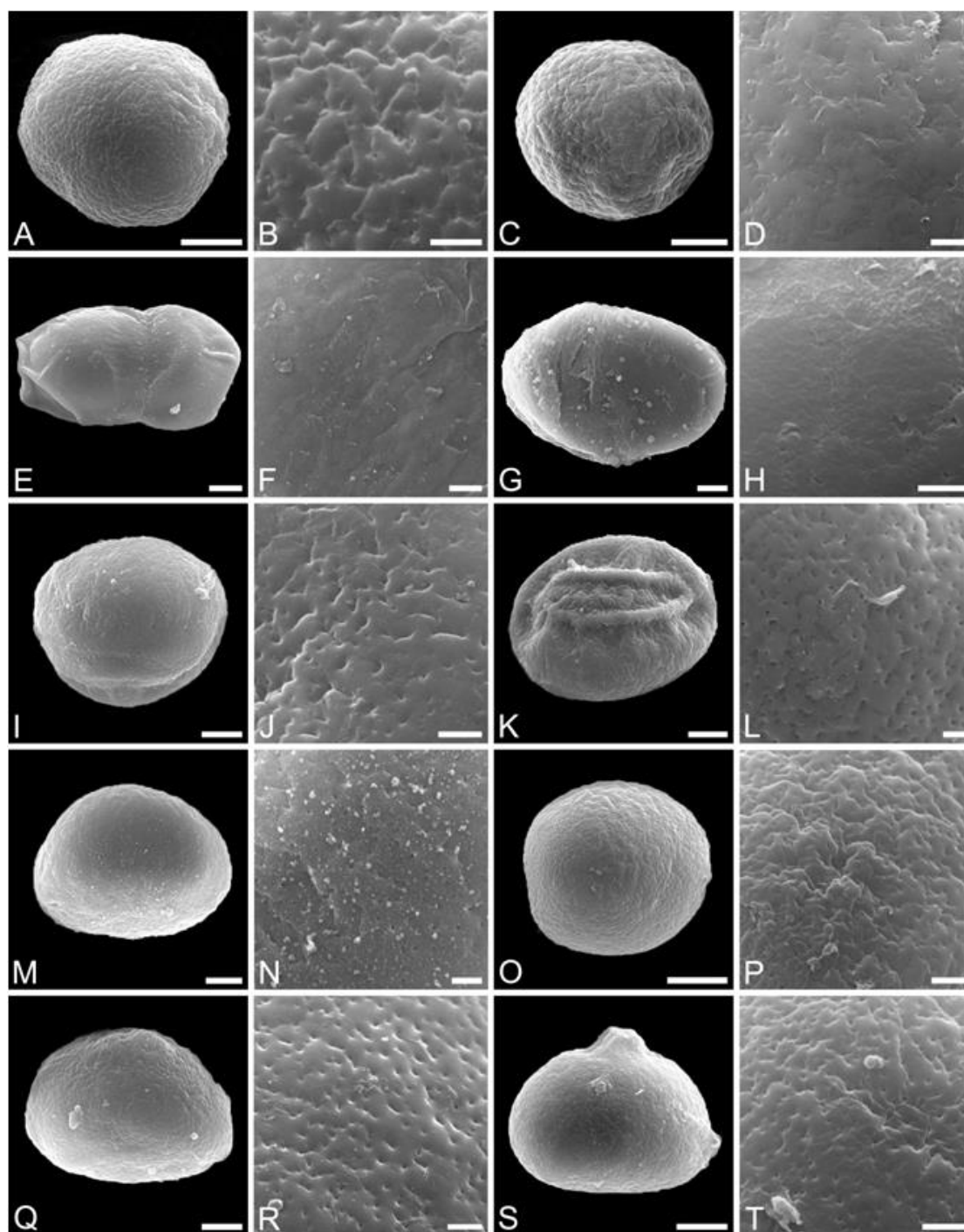
**Figure captions**

Figure 1. SEM views of pollen grains (**A, C, E, G, I, K, M, O, Q, S**) and ornamentation of sexine (**B, D, F, H, J, L, N, P, R, T**). **A, B.** *Alphonsea pallida*, spheroidal monad (**A**) with rugulate ornamentation (**B**). **C, D.** *Alphonsea siamensis*, spheroidal monad (**C**) with rugulate ornamentation (**D**). **E, F.** *Anaxagorea javanica*, intine extruded from aperture, exinous margin of aperture pointed out by black arrows (**E**), and with psilate exine (e) ornamentation (**F**). **G, H.** *Anaxagorea luzonensis*, intine

(i) extruded from aperture, exinous margin of aperture pointed out by black arrows (**G**), and with scabrate exine (e) ornamentation (**H**). **I, J.** *Artabotrys brevipes*, elliptic monad (**I**) with loosely fossulate-perforate ornamentation (**J**). **K, L.** *Artabotrys burmanicus*, elliptic monad with a single furrow (**K**), and with finely fossulate-perforate ornamentation (**L**). **M, N.** *Artabotrys harmandii*, elliptic monad (**M**) with loosely fossulate-perforate ornamentation (**N**). **O, P.** *Artabotrys multiflorus*, elliptic monad (**O**) with finely fossulate-perforate ornamentation (**P**). **Q, R.** *Artabotrys siamensis*, elliptic monad (**Q**) with finely fossulate-perforate ornamentation (**R**). **S, T.** *Artabotrys uniflorus*, elliptic monad (**S**) with finely fossulate-perforate ornamentation (**T**). Scale bars – 10 μm (A, C, E, G, I, K, M, O, Q, S), 2 μm (B, D, F, H, J, L, N, P, R, T).

Figure 2. SEM views of pollen grains (**A, C, E, G, I, K, M, O, Q, S**) and ornamentation of sexine (**B, D, F, H, J, L, N, P, R, T**). **A, B.** *Dasymaschalon acuminatum*, spheroidal monad (**A**) with echinate ornamentation and much smaller, closely spaced clavae (**B**). **C, D.** *Dasymaschlon angustifolium*, spheroidal monad (**C**) with echinate ornamentation and smaller, closely spaced clavae (**D**). **E, F.** *Dasymaschalon dasymaschalum*，spheroidal monad (**E**) with scabrate ornamentation (**F**). **G, H.** *Dasymaschalon macrocalyx*, spheroidal monad (**G**) with echinate ornamentation with many shallows (**H**). **I, J.** *Desmos cochinchinensis*, spheroidal monad (**I**) with echinate ornamentation (**J**). **K, L.** *Fissistigma oblongum*, elliptic monad (**K**) with a flattened pole (white arrow), and with rugulate ornamentation (**L**). **M, N.** *Friesodielsia desmoides*, spheroidal monad (**M**) with echinate-perforate ornamentation (**N**). **O, P.** *Goniothalamus laoticus*, tetrahedral tetrad (three grains of a tetrahedral tetrad) (**O**) with psilate ornamentation (**P**). **Q, R.** *Goniothalamus repevensis*, decussate tetrad (**Q**) with psilate-perforate ornamentation (**R**). **S, T.** *Goniothalamus sawtehii*, tetragonal tetrad (**S**) with rugulate ornamentation (**T**). Scale bars – 10 μm (A, C, E, G, I, K, M, O, Q, S), 2 μm (B, D, F, H, J, L, N, P, R, T).

Figure 3. SEM views of pollen grains (**A, C, E, G, I, K, M, O, Q–T**) and

ornamentation of sexine (**B, D, F, H, J, L, N, P**). **A, B.** *Goniothalamus tamirensis*, tetragonal tetrad (**A**) with psilate ornamentation (**B**). **C, D.** *Goniothalamus undulatus*, tetragonal tetrad (**C**) with scabrate ornamentation (**D**). **E, F.** *Huberantha cerasoides*, spheroidal monad with two depressed areas (**E**), rugulate-verrucate ornamentation (**F**). **G, H.** *Marsypopetalum crassum*, elliptic monad with two furrows (**G**), and with shallowly verrucate ornamentation (**H**). **I, J.** *Melodorum fruticosum*, elliptic monad (**I**) with scabrate-perforate ornamentation (**J**). **K, L.** *Miliusa campanulata*, spheroidal monad with two depressed areas (arrows) (**K**), with verrucate-perforate ornamentation (**L**). **M, N.** *Miliusa cuneata*, spheroidal monad with two depressed areas (white arrows) (**M**), with verrucate-perforate ornamentation (**N**). **O, P.** *Miliusa intermedia*, spheroidal monad with two depressed areas (white arrows) (**O**), with scabrate ornamentation (**P**). **Q–T.** *Mitrephora alba*, tetragonal (**Q**), rhomboidal (**R**), tetrahedral (**S**) and decussate (**T**) tetrad. Scale bars – 10 μm (A, C, E, G, I, K, M, O, Q–T), 2 μm (B, D, F, H, J, L, N, P).

Figure 4. SEM views of pollen grains (**B–E, G–I, K–M, O–S**) and ornamentation of sexine (**A, F, J, N, T**). **A.** *Mitrephora alba* with verrucate ornamentation. **B–F.** *Mitrephora keithii*, tetragonal (**B**), rhomboidal (**C**), tetrahedral (**D**) and new tetrad type (**E**) with verrucate ornamentation (**F**). **G–J.** *Mitrephora sp.*, tetragonal (**G**), rhomboidal (**H**) and tetrahedral (**I**) tetrad with verrucate ornamentation (**J**). **K–N.** *Mitrephora teysmanii*, tetragonal (**K**), rhomboidal (**L**) and tetrahedral (**M**) tetrad with verrucate ornamentation (**N**). **O–T.** *Mitrephora tomentosa*, tetragonal (**O**), rhomboidal (**P**), T-shaped (**Q**), tetrahedral (**R**) and decussate: two pairs of dyads across each other at right angles (arrows) (**S**) tetrad with verrucate ornamentation (**T**). Scale bars – 10 μm (B–E, G–I, K–M, O–S), 2 μm (A, F, J, N, T).

Figure 5. SEM views of pollen grains (**A–C, E–G, I, K, M, O, Q, S**) and ornamentation of sexine (**D**, **H**, **J, L, P, N, P, R, T**). **A–D.** *Mitrephora wangii*, tetragonal (**A**), rhomboidal (**B**) and decussate (**C**) tetrad with coarsely scabrate ornamentation (**D**). **E–H.** *Mitrephora winitii*, tetragonal (**E**), rhomboidal (**F**) and

tetrahedral (**G**) tetrad with verrucate ornamentation (**H**). **I, J.** *Monoon asteriellum*, spheroidal monad (**I**) with microechinate ornamentation (**J**). **K, L.** *Monoon fuscum*, spheroidal monad (**K**) with scabrate ornamentation (**L**). **M, N.** *Monoon jucundum*, spheroidal monad (**M**) with rugulate ornamentation (**N**). **O, P.** *Monoon lateriflorum*, spheroidal monad (**O**) with rugulate ornamentation (**P**). **Q, R.** *Monoon membranifolium*, spheroidal monad (**Q**) with scabrate ornamentation (**R**). **S, T.** *Monoon viride*, spheroidal monad (**S**) with rugulate ornamentation (**T**). Scale bars – 10 μm (A–C, E–G, I, K, M, O, Q, S), 2 μm (D, H, J, L, P, N, P, R, T).

Figure 6. SEM views of pollen grains (**A, C, E, G, I, J, L, N, O, Q, S**) and ornamentation of sexine (**B, D, F, H, K, M, P, R, T**). **A, B.** *Orophea brandisii*, spheroidal monad with two furrow (**A**), and with verrucate ornamentation (**B**). **C, D.** *Orophea enterocarpa*, spheroidal monad with two furrows (**C**), and with verrucate ornamentation (**D**). **E, F.** *Orophea kerrii*, monad with two furrows (**E**), and with rugulate ornamentation (**F**). **G, H.** *Orophea polycarpa*, monad with one visible furrow (**G**), and with reticulate-perforate ornamentation (**H**). **I–K.** *Polyalthia bullata*, triangular monad with two furrows (**I, J**) with rugulate ornamentation (**K**). **L, M.** *Polyalthia evecta* var. *evecta*, spheroidal monad with one furrow (**L**) with verrucate ornamentation (**M**). **N–P.** *Polyalthia evecta* var. *attopeuernsis*, triangular monad (**N, O**) with rugulate-verrucate ornamentation (**P**). **Q, R.** *Uvaria siamensis*, spheroidal monad (**Q**) with gemmate ornamentation (**R**). **S, T.** *Winita expansa*, elliptic monad with no visible aperture (**S**), and with microscabrate ornamentation (**T**). Scale bars – 10 μm (A, C, E, G, I, J, L, N, O, Q, S), 2 μm (B, D, F, H, K, M, P, R, T).